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(12) **United States Patent**
Borca et al.(10) **Patent No.:** **US 9,463,234 B2**
(45) **Date of Patent:** **Oct. 11, 2016**(54) **ATTENUATED AFRICAN SWINE FEVER VIRUS STRAIN INDUCES PROTECTION AGAINST CHALLENGE WITH HOMOLOGOUS VIRULENT PARENTAL VIRUS GEORGIA 2007 ISOLATE**(71) Applicants: **The United States of America, as Represented by the Secretary of Agriculture**, Washington, DC (US); **The University of Connecticut**, Storrs, CT (US)(72) Inventors: **Manuel V. Borca**, Westbrook, CT (US); **Lauren G. Holinka-Patterson**, Deep River, CT (US); **Douglas Gladue**, Guildford, CT (US); **Guillermo R. Risatti**, Westbrook, CT (US); **Vivian K. O'Donnell**, Old Saybrook, CT (US)(73) Assignees: **The United States of America, as represented by the Secretary of Agriculture**, Washington, DC (US); **University of Connecticut**, Farmington, CT (US)

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(52) **U.S. Cl.**

CPC **A61K 39/12** (2013.01); **C12N 7/00** (2013.01); **C12Q 1/701** (2013.01); **G01N 33/56983** (2013.01); **A61K 2039/552** (2013.01); **C12N 2710/12021** (2013.01); **C12N 2710/12034** (2013.01); **C12Q 2600/158** (2013.01); **G01N 2333/01** (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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McKillen et al. (Journal of Virological Methods. Jun. 2010; 168: 141-146).^{*}

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(57) **ABSTRACT**

African swine fever virus (ASFV) is the etiological agent of a contagious and often lethal viral disease of domestic pigs that has significant economic consequences for swine breeding. Control of ASF has been hampered by the unavailability of vaccines. Recombinant viruses harboring engineered deletions of specific virulence-associated genes induce solid protection against the challenge with parental viruses. Here we report the construction of a recombinant Δ9GL virus derived from the highly virulent ASFV Georgia 2007 (ASFV-G) isolate. In vivo, ASFV-G Δ9GL administered intramuscularly (IM) to swine at relatively high doses (10^4 HAD₅₀) retains a virulent phenotype practically indistinguishable from the parental virus. Conversely, at low IM doses (10^2 or 10^3 HAD₅₀), ASFV-G Δ9GL does not induce disease. Importantly, animals infected with 10^3 HAD₅₀ are protected against the presentation of clinical disease when challenged at 28 days post infection with the virulent parental strain Georgia 2007.

4 Claims, 3 Drawing Sheets

| | SEQ ID NO: | 10 | 20 | 30 | 40 | 50 | 60 |
|------------------------|------------------|-------------|------------|------------|------------|------------|------------|
| ASFV-G | 2 | MLHWCPKYWR | SLHLYAIFFS | DAPSWKEKYE | AIQWILNFIE | SLPCIRCQHH | AFSYLTKNPL |
| Malawi L1l-20/1 | 17 |f.. | T..... | T.G..... | | M.R..... | |
| Pr4 | 18 |A..... | G..... | | | R..... | |
| Pr5 | 18 |A..... | ...G..... | | | R..... | |
| Killean3 | 19 |F.. | A..... | N..... | | M.R..... | |
| Cr1 | 18 |A..... | ...G..... | | | R..... | |
| Cr3 | 18 |A..... | ...G..... | | | R..... | |
| Tengani(62) | 20 |A..... | T.G..... | | | | |
| Fairfield/96/1 | 21 |A..... | G..... | | | | |
| Wildebeeslaagte | 18 |A..... | ...C..... | | | R..... | |
| Kimakia(64) | 22 | | | | | R..... | |
| Victoria Falls | 23 | | | | S..... | | |
| Zaire (67) | 2 | | | | | | |
| Uganda(61) | 2 | | | | | | |
| E70 | 2 | | | | | | |
| E75 | 2 | | | | | | |
| Haiti | 2 | | | | | | |
| Spencer(51) | 2 | | | | | | |
| La Granja(63) | 2 | | | | | | |
| Lisbon(60) | 2 | | | | | | |
| Lee(55) | 2 | | | | | | |
| | | 70 | 80 | 90 | 100 | 110 | 120 |
| ASFV-G | 2 | TLNNSEDEDY | WTFAFHNNVN | NRLNKKIISW | SEYKNIYEQS | ILKTIEYGKT | DFIGAWSSI* |
| Malawi L1l-20/1 | 17 | | | K..... | | N..... |* |
| Pr4 | 18 | | | | | |* |
| Pr5 | 18 | | | | | |* |
| Killean3 | 19 | | | K..... | | |* |
| Cr1 | 18 | | | | | |* |
| Cr3 | 18 | | | | | |* |
| Tengani(62) | 20 | | | | | |* |
| Fairfield/96/1 | 21 | | | | | |* |
| Wildebeeslaagte | 18 | | | | | |* |
| Kimakia(64) | 22 | | | | | |* |
| Victoria Falls | 23 | | | | | |* |
| Zaire (67) | 2 | | | | | |* |
| Uganda(61) | 2 | | | | | |* |
| E70 | 2 | | | | | |* |
| E75 | 2 | | | | | |* |
| Haiti | 2 | | | | | |* |
| Spencer(51) | 2 | | | | | |* |
| La Granja(63) | 2 | | | | | |* |
| Lisbon(60) | 2 | | | | | |* |
| Lee(55) | 2 | | | | | |* |

Fig. 1

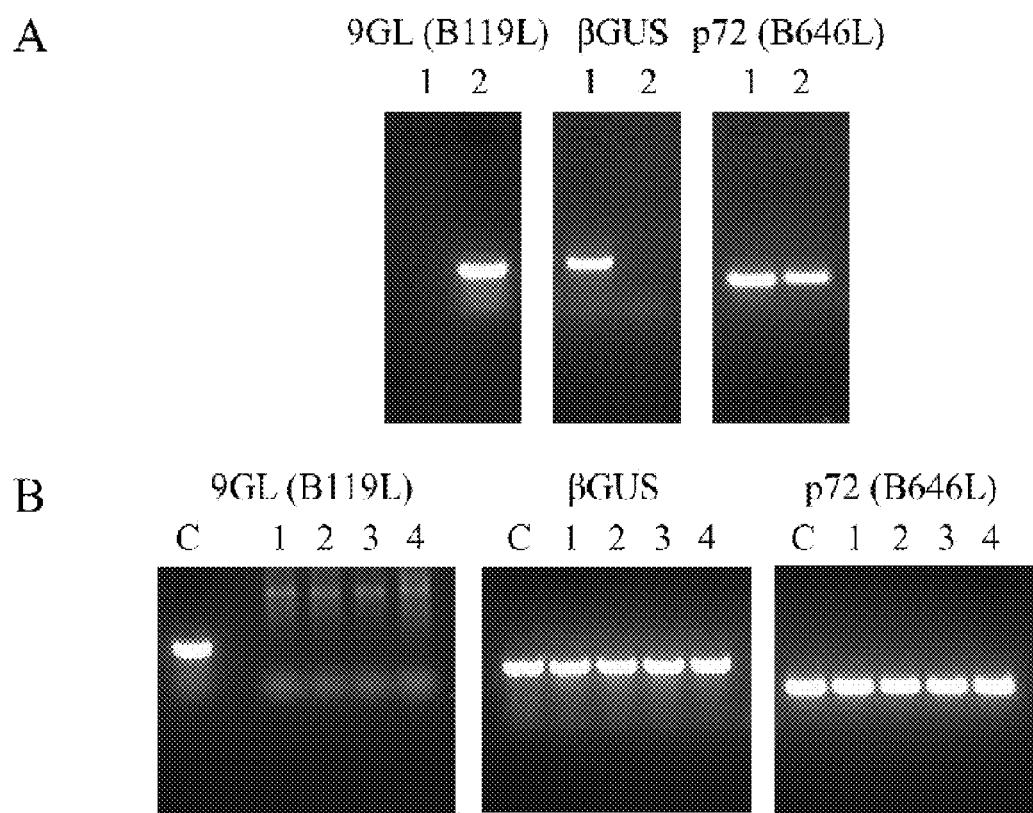


Fig. 2

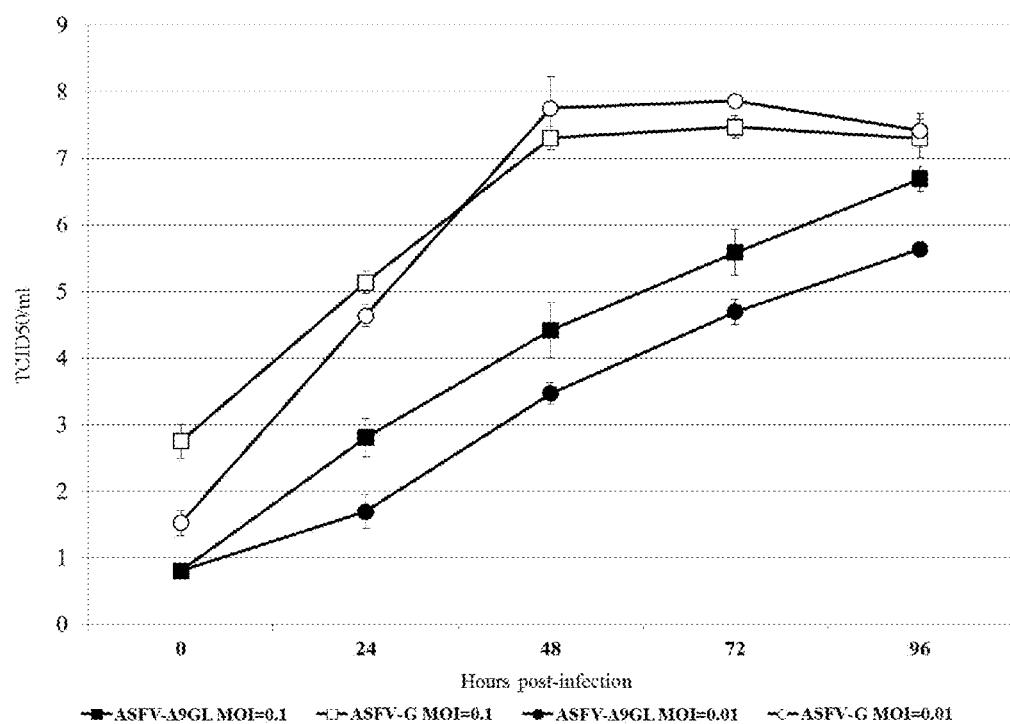


Fig. 3

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**ATTENUATED AFRICAN SWINE FEVER
VIRUS STRAIN INDUCES PROTECTION
AGAINST CHALLENGE WITH
HOMOLOGOUS VIRULENT PARENTAL
VIRUS GEORGIA 2007 ISOLATE**

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the construction of a recombinant African Swine Fever Virus (ASFV) live attenuated candidate strain vaccine for the highly virulent Georgia 2007 isolate ASFV-G. The vaccine comprises the ASFV-G Δ9GL, a recombinant ASFV-G modified by deleting a large portion of the 9GL gene.

2. Description of the Relevant Art

African Swine Fever (ASF) is a contagious viral disease of swine. The causative agent, ASF virus (ASFV), is a large enveloped virus containing a double-stranded DNA genome of approximately 190 kilobase pairs. ASFV shares aspects of genome structure and replication strategy with other large double-stranded DNA viruses, including the Poxviridae, Iridoviridae and Phycodnaviridae (Costard et al. 2009. *Phil. Trans. Royal Soc. B* 364:2683-2696). ASFV infections in domestic pigs are often fatal and are characterized by fever, hemorrhages, ataxia and severe depression. However, the course of infection varies, ranging from highly lethal to sub-clinical, depending on host characteristics and the particular virus strain (Tulman et al. 2009. *Curr. Top. Microbiol. Immunol.* 328:43-87).

Currently, the disease is endemic in more than twenty sub-Saharan African countries. In Europe, ASF is still endemic on the island of Sardinia (Italy) and new outbreaks have been declared in the Caucasus region since 2007, affecting Georgia, Armenia, Azerbaijan and Russia. Isolated outbreaks have been recently reported in Ukraine, Belarus, Lithuania, Latvia and Poland, posing the risk of further dissemination into neighbouring countries. The epidemic virus, ASFV Georgia 2007/1, is a highly virulent isolate belonging to the genotype II (Chapman et al. 2011. *Emerging Infect. Dis.* 17:599-605).

At present, there is no vaccine available for ASF and disease outbreaks are controlled by animal quarantine and slaughter. Attempts to vaccinate animals using infected cell extracts, supernatants of infected pig peripheral blood leukocytes, purified and inactivated virions, infected glutaraldehyde-fixed macrophages, or detergent-treated infected alveolar macrophages failed to induce protective immunity (Coggins, L. 1974. *Prog. Med. Virol.* 18:48-63; Forman et al. 1982. *Arch. Virol.* 74:91-100; Kihm et al. 1987. In: *African Swine Fever*, Becker, Y. (ed), Martinus Nijhoff, Boston, pp 127-144; Mebus, C. A. 1988. *Adv. Virus Res.* 35:251-269). Homologous protective immunity does develop in pigs surviving viral infection. Pigs surviving acute infection with moderately virulent or attenuated variants of ASFV develop long-term resistance to homologous, but rarely to heterologous, virus challenge (Hamdy and Dardiri. 1984. *Am. J. Vet. Res.* 45:711-714; Ruiz-Gonzalvo et al. 1981. In: *FAO/CEC Expert Consultation in ASF Research*, Wilkinson, P. J. (ed), Rome, pp 206-216). Pigs immunized with live attenuated ASF viruses containing engineered deletions of specific ASFV virulence-associated genes were protected when challenged with homologous parental virus. Specifically, individual deletion of UK (DP69R), 23-NL (DP71L), TK (A240L) or 9GL (B119L) genes from the genomes of pathogenic ASF viruses (Malawi Lil-20/1, Pretoriuskop/96/4, and E70) markedly attenuated the virus in swine and the animals immunized with these attenuated viruses were protected against challenge with homologous virus (Moore et

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al. 1998. *J. Virol.* 72:10310-10315; Lewis et al. 2000. *J. Virol.* 74:1275-1285; Zsak et al. 1996. *J. Virol.* 70:8865-8871; Zsak et al. 1998. *J. Virol.* 72:1028-1035). In particular, deletion of 9GL (B119L) in highly virulent ASFV isolates Malawi Lil-20/1 and Pretoriuskop/96/4 resulted in complete attenuation of these viruses in swine (Lewis et al., supra; Neilan et al. 2004. *Virol.* 319:337-342). Administration of Malawi Lil-20/1 or Pretoriuskop/96/4 Δ9GL mutants to pigs via IM injection at a relatively high virus dose (10^4 HAD₅₀ [50% hemadsorbing dose]) did not induce clinical signs, with all animals surviving the infection. Furthermore, IM inoculation of pigs with these viruses even at a relatively low dose (10^2 HAD₅₀) induced protection against challenge with virulent Malawi Lil-20/1 virus (Lewis et al., supra). These observations constitute the only experimental evidence describing the rational development of an effective live attenuated virus against ASFV.

Although a deletion within the 9GL (B119L) gene from ASF Malawi Lil-20/1 and Pretoriuskop/96/4 and E70 had resulted in attenuated viruses effective for protection from pathogenic parental ASFV, the modified viruses did not protect against heterologous ASFV strains. Thus, there is a need for an effective live attenuated vaccine for the highly virulent ASFV Georgia 2007 isolate, ASFV-G, for which there is no vaccine candidate.

SUMMARY OF THE INVENTION

We have developed the novel recombinant mutant ASFV-G Δ9GL virus, a modification of the ASFV-G (African Swine Fever Virus-Georgia 2007 isolate).

In accordance with this discovery, it is an object of the invention to provide the novel mutant ASFV-G Δ9GL virus, resulting from the deletion of a large portion of the 9GL gene of the parental ASFV-G. The nucleotide sequence of ASFV-G Δ9GL (SEQ ID NO:3) differs from the nucleotide sequence encoding the ASFV-G (SEQ ID NO:1). While the nucleotide sequence of ASFV-G (SEQ ID NO:1) encodes the wild-type 9GL (B119L) protein of 119 amino acids (SEQ ID NO:2), the nucleotide sequence of ASFV-G Δ9GL (SEQ ID NO:3) encodes a mutant 9GL polypeptide of 58 amino acids (SEQ ID NO:4) resulting from the deletion of amino acid #11 through amino acid #68 of the wild-type 9GL polypeptide (SEQ ID NO:2) of ASFV-G.

An added object of the invention is to provide immunogenic compositions comprising a viable ASFV-G Δ9GL virus.

An additional object of the invention is to provide a rationally designed live attenuated ASFV-G Δ9GL vaccine effective to protect an animal from clinical ASF disease when challenged with pathogenic ASFV-G.

A further object of the invention is to provide a genetic marker vaccine which can potentially distinguish between vaccinated animals and animals infected with ASFV-G.

Another object of the invention is to provide a method for protecting an animal against ASFV-G by administering an effective amount of rationally designed live attenuated ASFV-G Δ9GL vaccine.

An additional object of the invention is to provide a method for distinguishing animals infected with ASFV-G from animals vaccinated with said rationally designed live attenuated ASFV-G Δ9GL vaccine, comprising a genetic DIVA strategy for differentiating vaccinated animals from wild-type infected animals.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the sequence alignment of 9GL (B119L) gene encoded proteins by ASFV. The nucleotide sequence of

ASFV-G (SEQ ID NO: 1) encodes the wild-type 9GL (B119L) protein of 119 amino acids (SEQ ID NO: 2, shown). Isolates of various temporal and geographic origins, including those from obtained from ticks and pig sources, were compared and are identified by SEQ ID Nos: 2 and 17-23. The partial deletion introduced into ASFV-G that yielded ASFV-G Δ9GL virus is shown between brackets.

FIGS. 2A and 2B depict PCR analysis of ASFV-G Δ9GL virus DNA using specific primers targeting 9GL (B119L), p72 (B646L), or βGus genes. FIG. 2A depicts an assessment of purity of the ASFV-G Δ9GL virus stock by PCR. Lane 1: ASFV-G Δ9GL; Lane 2: ASFV-G. FIG. 2b depicts identification of the presence of parental ASFV-G in viruses (V1-V4) isolated from animals infected with ASFV-G Δ9GL virus. Control (C) consists of a plasmid containing the respective target genes.

FIG. 3 shows the in vitro growth kinetics of ASFV-G Δ9GL and parental ASFV-G viruses. Primary swine macrophage cell cultures were infected (MOI=0.1 or 0.01) with either ASFV-G Δ9GL or parental ASFV-G viruses and virus yield obtained at the indicated times post-infection were titrated in primary swine macrophage cell cultures. Data represent means and standard deviations from two independent experiments. Sensitivity of virus detection: ≥1.8 TCID₅₀/ml.

DETAILED DESCRIPTION OF THE INVENTION

We have developed an attenuated virus that can be used as a vaccine candidate through the approach of targeting 9GL (B119L) for genetic modifications. Here we report the construction of a recombinant Δ9GL virus of the highly virulent ASFV Georgia 2007 isolate (ASFV-G). In vitro, as observed with Δ9GL viruses Malawi Lil-20/1 and Pretoriuskop/96/4, ASFV-G Δ9GL has a decreased ability, relative to the parental virus, to replicate in swine macrophage primary cultures. ASFV-G Δ9GL administered intramuscularly (IM) to swine at relatively high doses (10⁴ HAD₅₀) retains a virulent phenotype indistinguishable from the parental virus. Intramuscular inoculation of a low dose (10² or 10³ HAD₅₀) of ASFV-G Δ9GL does not induce disease. Animals infected with 10³ HAD₅₀ are protected against the presentation of clinical disease when challenge at 28 days post infection with the virulent parental strain Georgia 2007.

No vaccines are available to prevent ASFV infection. Only live attenuated virus strains have been useful in protecting pigs against challenge with homologous virulent isolates. These attenuated viruses have been regularly produced by sequential passages in cell cultures and, more recently, by genetic manipulation. Naturally occurring attenuated viruses have been used as live vaccine candidates. Attenuated viruses obtained by genetic manipulation involve the deletion of specific genes by a process of homologous recombination. Independent deletion of four different genes from ASFV has been shown to attenuate virulent viruses. Independent deletions of the NL (DP71 L) (Zsak et al. 1996, supra) or the UK (DP69R) (Zsak et al. 1998, supra) genes from ASFV E75, deletion of the TK (A240L) gene (Moore et al., supra) from ASFV adapted to Vero cells, Malawi Lil-20/1 and Haiti, and deletion of the 9GL (B119L) gene also from Malawi Lil-20/1 (Lewis et al., supra) and Pretoriuskop/96/4 (Neilan et al., supra) isolates rendered recombinant deletion mutant viruses with significantly reduced virulence in swine. In all these cases, animals inoculated with each of these genetically modified viruses survived the infection and became protected against ASFV when challenged with the corresponding virulent parental virus (homologous challenge) (Lewis et al., supra; Moore et al., supra; Neilan et al., supra; Zsak et al. 1996, supra; Zsak

et al. 1998, supra). Those findings suggest that development of attenuated ASFV recombinant viruses by genetic manipulations of target genes is an effective approach for vaccine development.

The NL (DP71L) gene product exists in two different forms, a long (184 amino acids as in 23-NL) or a short form (70 to 72 amino acids) depending on the ASFV isolate (Zsak et al. 1996, supra). Although deletion of this gene in ASFV E70 isolate (short form) rendered an attenuated virus, the deletion of the NL (DP71L) gene from ASFV Malawi Lil-20/1 (long form) or Pretoriuskop/96/4 (short form) did not result in attenuation of the virus (Afonso et al. 1998. *J. Gen. Virol.* 79 (Pt. 10):2543-2547). Deletion of the TK (A240L) gene, a highly conserved gene among all ASFV isolates involved in DNA synthesis, has been introduced into pathogenic Vero cell-adapted Malawi Lil-20/1 and Haiti H811 viruses. The Malawi Lil-20/1 mutant virus was less virulent in vivo than the revertant virus (wild-type-like virus) but it was not completely attenuated (Moore et al., supra). The UK (DP69R) gene is located in the right variable region of certain ASFV isolates. Deletion of this gene from ASFV E70 isolates rendered a virus exhibiting reduced virulence (Zsak et al. 1998, supra). Although the UK (DP69R) gene is conserved, it is not present in every ASFV isolate (e.g. Malawi Lil-20/1), limiting its use as a candidate target gene for producing attenuated viruses.

The 9GL (B119L) gene is highly conserved among ASFV isolated and sequenced so far, including those from both tick and pig sources. The fact that deletion of the gene from virulent Malawi Lil-20/1 (Lewis et al., supra) and Pretoriuskop/96/4 (Neilan et al., supra) effectively reduced virulence in swine and induced protection makes 9GL (B119L) a strong candidate target gene for modification and production of attenuated virus that can confer effective protection against ASFV. Interestingly, here we observed that deletion of 9GL (B119L) from the ASFV-G isolate does not have the same effect in terms of attenuation and protection as reported for Malawi Lil-20/1 and Pretoriuskop/96/4. Only when ASFV-G Δ9GL was administrated at low dose to swine was it possible to observe a decrease in virus virulence. Data presented here indicate that the 9GL (B119L) gene is not required for ASFV-G virulence and that other virulence factors may be involved in the process. As observed with deletions of NL (DP71L) in E70, Malawi Lil-20/1 and Pretoriuskop/96/4 isolates that lead to different phenotypes (Zsak et al. 1996, supra; Afonso et al., supra), deletions of 9GL (B119L) have produced similar outcomes, suggesting that virulence of ASFV is the result of a multigene effect.

The NL proteins encoded by E70 (short form) and Malawi Lil-20/1 (long form) differ significantly and that may explain the phenotypic differences observed in swine inoculated with the respective deletion mutant viruses. However, protein identity matrixes indicate that the 9GL protein is highly similar among ASFV isolates where ASFV-G, Malawi Lil-20/1, and Pretoriuskop/96/4 share over 93% amino acid identity, making it unlikely that ASFV attenuation relies solely on protein divergence. Since the observed phenotypes are most likely mediated by the effect of multiple genes (Lewis et al., supra; Moore et al., supra; Neilan et al., supra; Zsak et al. 1996, supra; Zsak et al. 1998, supra), the evidence accumulated so far makes it difficult to speculate what is indeed the spectrum of genes mediating virulence in the ASFV Georgia 2007 isolate.

Nevertheless, a sub-lethal dose of ASFV-G Δ9GL is able to induce effective protection against the presentation of clinical disease after the challenge with homologous parental virus.

In summary, here we present evidence that deletion of 9GL (B119L), a gene that has been associated with virus virulence in Malawi Lil-20/1 and Pretoriuskop/96/4 isolates,

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does not drastically alter virulence of ASFV-G, i.e., inoculation of 10^4 HAD₅₀ of ASFV-G Δ9GL caused disease. Although, when used at sub-lethal doses, ASFV-G Δ9GL entirely protects pigs against challenge with the virulent parental ASFV-G.

A vaccine is defined herein as a biological agent which is capable of providing a protective response in an animal to which the vaccine has been delivered and is incapable of causing severe disease. Administration of the vaccine results in immunity from a disease; the vaccine stimulates antibody production or cellular immunity against the pathogen causing the disease. Immunity is defined herein as the induction of a significant higher level of protection in a population of swine against mortality and clinical symptoms after vaccination compared to an unvaccinated group. In particular, the vaccine according to the invention protects a large proportion of vaccinated animals against the occurrence of clinical symptoms of the disease and mortality. The vaccine of the invention herein is a genetically engineered mutant virus vaccine. A genetic marker vaccine is defined as a vaccine that, in conjunction with a diagnostic test, enables genetic differentiation of vaccinated animals from infected animals. A deletion mutation can be used to differentiate infected from vaccinated animals as is observed in Example 7—Genetic DIVA Strategy—Real-Time RT-PCR. A mutation is understood to be a change in the genetic information of a “wild-type” or unmodified 9GL (B119L) gene of a parent ASFV-G strain which is able to express native 9GL proteins. Thus, the 9GL polypeptide expressed by the ASFV-G Δ9GL mutant virus is changed: the 9GL protein from ASFV-G Δ9GL has fewer amino acids than the wild-type 9GL, as amino acids #11 through #68 are deleted in the 9GL polypeptide of ASFV-G Δ9GL. The ASFV-G Δ9GL recombinant ASFV-G mutant comprising DNA encoding a mutation in the ASFV-G 9GL protein, wherein the mutation comprises a deletion of 58 amino acids, wherein said recombinant ASFV-G mutant (ASFV-G Δ9G) is a live attenuated ASFV-G vaccine when used at IM inoculation doses of 10^3 HAD₅₀.

A vaccine against ASFV-G is provided that comprises a ASFV-G Δ9G virus mutant as defined above in a live form, and a pharmaceutically acceptable carrier or diluent. The vaccine according to the invention containing the live virus can be prepared and marketed in the form of a suspension or in a lyophilized form and additionally contains a pharmaceutically acceptable carrier or diluent customary used for such compositions. Carriers include stabilizers, preservatives and buffers. Suitable stabilizers are, for example SPGA (sucrose, phosphate, glutamate, and human albumin), carbohydrates (such as sorbitol, mannitol, starch, sucrose, dextran, glutamate or glucose), proteins (such as dried milk serum, albumin or casein) or degradation products thereof. Suitable buffers are for example alkali metal phosphates. Suitable preservatives are thimerosal, merthiolate and gentamicin. Diluents include water, aqueous buffer (such as buffered saline), alcohols and polyols (such as glycerol).

If desired, the live vaccines according to the invention may contain an adjuvant. Examples of suitable compounds and compositions with adjuvant activity are well known in the art. Furthermore, nucleic acid sequences encoding polypeptides for pharmaceutical or diagnostic applications, in particular immunomodulators such as lymphokines, interferons or cytokines, may be incorporated into the vaccine.

A vaccine according to the invention can be prepared by conventional methods such as those commonly used for the commercially available live attenuated ASFV vaccines. Briefly, a susceptible substrate is inoculated with the

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ASFV-G Δ9GL mutant and propagated until the virus has replicated to a desired titer after which ASFV-G Δ9GL-containing material is harvested. Subsequently, the harvested material is formulated into a pharmaceutical preparation with immunizing properties.

Every substrate which is able to support the replication of ASFV-G Δ9GL viruses can be used in the present invention, including primary cultures of swine peripheral blood macrophages.

The vaccine may be administered by intramuscular, subcutaneous or intranasal inoculation or injection in an amount which is effective to protect the animal against challenge by a virulent strain of ASFV-G. This amount may vary according to the animal being inoculated, taking into consideration the size and weight of the animal. The vaccine according to the invention comprises an effective dosage of the ASFV-G Δ9GL mutant as the active component, i.e. an amount of immunizing ASFV-G Δ9GL material that will induce immunity in the vaccinated animals, swine, against challenge by a virulent ASFV-G. Immunity is defined herein as the induction of a significant higher level of protection in a population of swine against mortality and clinical symptoms after vaccination compared to an unvaccinated group. In particular, the vaccine according to the invention prevents a large proportion of vaccinated animals against the occurrence of clinical symptoms of the disease and mortality. Typically, the live vaccine can be administered in a dose of 10^3 HAD₅₀. Effective amounts may be experimentally determined as necessary by those of skill in the art by following the guidance provided, for example, by Example 6.

In addition to the ASFV-G Δ9GL mutant, the invention can also include combination vaccines comprising a vaccine strain capable of inducing protection against another porcine pathogen.

The ASFV-G Δ9GL vaccine described above, in conjunction with a diagnostic method, has the potential of distinguishing between animals that are vaccinated with it and animals that are infected with naturally occurring ASFV-G strains or vaccinated with conventional ASFV-G vaccines.

The present invention also provides an invaluable tool to monitor ASFV-G control measures that may lead to eradication of ASFV-G if applied in large scale stamping out programs. This tool concerns a method for determining ASFV-G infection in swine comprising the step of examining a sample of the animal for the presence of nucleotides encoding the wild-type ASFV-G 9GL protein versus the polynucleotide encoding the shorter ASFV-G Δ9GL polypeptide due to deletions in the 9GL gene of ASFV-G Δ9GL. The sample of the animal used in this method may be any sample in which ASFV-G versus ASFV-G Δ9GL genetic differences allowing for differentiating of natural infection versus vaccination can be detected by genetic DIVA.

EXAMPLES

Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein only to further illustrate the invention and are not intended to limit the scope of the invention as defined by the claims.

Example 1

Cell Cultures and Viruses

Primary swine macrophage cell cultures were prepared from defibrinated swine blood as previously described by

Zsak et al. (1996, *supra*). Briefly, heparin-treated swine blood was incubated at 37° C. for 1 hour to allow sedimentation of the erythrocyte fraction. Mononuclear leukocytes were separated by flotation over a Ficoll-Paque (Pharmacia, Piscataway, N.J.) density gradient (specific gravity, 1.079). The monocyte/macrophage cell fraction was cultured in plastic Primaria (Falcon; Becton Dickinson Labware, Franklin Lakes, N.J.) tissue culture flasks containing macrophage media, composed of RPMI 1640 Medium (Life Technologies, Grand Island, N.Y.) with 30% L929 supernatant and 20% fetal bovine serum (HI-FBS, Thermo Scientific, Waltham, Mass.) for 48 hours at 37° C. under 5% CO₂. Adherent cells were detached from the plastic by using 10 mM EDTA in phosphate buffered saline (PBS) and were then reseeded into Primaria T25, 6- or 96-well dishes at a density of 5×10⁶ cells per ml for use in assays 24 hours later.

ASFV Georgia (ASFV-G) was a field isolate kindly provided by Dr. Nino Vepkhvadze, from the Laboratory of the Ministry of Agriculture (LMA) in Tbilisi, Republic of Georgia.

Comparative growth curves between ASFV-G and ASFV-G Δ9GL viruses were performed in primary swine macrophage cell cultures. Preformed monolayers were prepared in 24-well plates and infected at MOI of 0.01 (based on HAID₅₀ previously determined in primary swine macrophage cell cultures). After 1 hour of adsorption at 37° C. under 5% CO₂ the inoculum was removed and the cells were rinsed two times with PBS. The monolayers were then rinsed with macrophage media and incubated for 2, 24, 48, 72 and 96 hours at 37° C. under 5% CO₂. At appropriate times post-infection, the cells were frozen at -70° C. and the thawed lysates were used to determine titers by HAID₅₀/ml in primary swine macrophage cell cultures. All samples were run simultaneously to avoid inter-assay variability.

Virus titration was performed on primary swine macrophage cell cultures in 96-well plates. Virus dilutions and cultures were performed using macrophage medium. Presence of virus was assessed by hemadsorption (HA) and virus titers were calculated by the Reed and Muench method (1938. *Amer. J. Hygiene* 27:493-497).

Example 2

Construction of the Recombinant 9GL (B119L) Gene Deletion Mutant of ASFV-G Isolate

ASFV-G Δ9GL was constructed from the highly pathogenic ASFV Georgia 2007 isolate (ASFV-G). Recombinant ASFVs were generated by homologous recombination between the parental ASFV genome and a recombination transfer vector following infection and transfection of swine macrophage cell cultures (Neilan et al., *supra*; Zsak et al. 1996, *supra*). Recombinant transfer vector (p72GUSAΔ9GL) contained flanking genomic regions, which included portions of 9GL mapping to the left (1.2 kbp) and right (1.15 kbp) of the gene and a reporter gene cassette containing the β-glucuronidase (GUS) gene with the ASFV p72 late gene promoter, p72GUS (45). A 173-bp region, encompassing amino acid residues 11 to 68, within the 9GL (B119L) gene (FIG. 1) was deleted from ASFV-G virus and replaced with a cassette containing the p72GUS reporter gene cassette by the homologous recombination. Recombinant transfer vector p72GUSAΔ9GL was obtained by DNA synthesis (GenScript, Piscataway, N.J., USA). Macrophage cell cultures were infected with ASFV-G and transfected with p72GUSAΔ9GL. The recombinant virus was obtained after 11 successive plaque purification events on monolayers of primary swine macrophage cell cultures. The virus popula-

tion obtained from the last round of plaque purification was amplified in primary swine macrophage cell cultures to obtain a virus stock.

The extent of purity of ASFV-G Δ9GL in the virus stock as well as in virus isolated from infected animals was assessed by PCR. To ensure the absence of parental ASFV-G, virus DNA was extracted from the virus stock and analyzed by PCR using primers targeting genes p72 (B646L), 9GL (B119L) and β-Gus. Detection of the 9GL (B119L) gene was performed using the following pair of primers: forward 5'TAGAGATGACCAGGCTCCAA3' (SEQ ID NO:5); reverse 5'GTTGCATTGGGGACCT AAATACT 3' (SEQ ID NO:6). Detection of the β-Gus gene was performed using the following pair of primers: forward 5'GACGGCCTGTGGCATT3' (SEQ ID NO:7); reverse 5'GCGATGGATTCCGGCAT3' (SEQ ID NO:8). Detection of the p72 (B646L) gene was performed using the following pair of primers: forward 5'GTCTTATTGCTAAC GATGGGAAG3' (SEQ ID NO:9); reverse 5'CCAAAGG-TAAGCTTGTCCCCTAA3' (SEQ ID NO:10).

PCR products were sequenced using the dideoxynucleotide chain-termination method (Sanger et al. 1977. *Proc. Natl. Acad. Sci. USA* 74:5463-5467). Sequencing reactions were prepared with the Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif.). Reaction products were sequenced on a PRISM 3730xl automated DNA sequencer (Applied Biosystems). Sequence data were assembled with the Phrap software program (Retrieved from the Internet: phrap.org), with confirmatory assemblies performed using CAP3 (Huang and Madan. 1999. *Genome Res.* 9:868-877). The final DNA consensus sequence represented an average five-fold redundancy at each base position. Sequence comparisons were conducted using BioEdit software (Tom Hall, Ibis Biosciences Carlsbad, Calif., Copyright 1997-2013).

Only amplicons for p72 (B646L) and β-Gus genes were detected in DNA extracted from the virus stock; whereas no amplicons were generated with primers targeting the 9GL (B119L) gene (FIG. 2A) indicating the lack of contamination of the ASFV-G Δ9GL stock with ASFV-G.

Example 3

Full Genome Sequence Analysis: ASFV-G Δ9GL Relative to Parental ASFV-G

To evaluate the accuracy of the genetic modification and the integrity of the genome of the recombinant virus, full genome sequences of ASFV-G Δ9GL and parental ASFV-G were obtained using Next Generation Sequencing (NGS) and compared. First, a full-length genome comparison between parental ASFV-G and ASFV Georgia 2007/1 (Chapman et al., *supra*) was performed. ASFV DNA was obtained from the cytoplasm of infected cells using the Trizol method (Life Technologies, Grand Island, N.Y., USA). DNA concentration was determined using the Qubit® dsDNA HS assay kit (Life Technologies) and read on a Qubit® 2 Fluorometer (Life Technologies). One microgram of virus DNA was enzymatically fragmented to obtain blunt end fragments in a length range of 200-300 bp using the Ion Shear™ Plus reagent kit (Life Technologies) and incubated at 37° C. in a Peltier Thermal Cycler DNA Engine Tetrad 2. After shearing, the fragmented DNA library was loaded onto a DNA chip (Agilent, Santa Clara, Calif., USA) and analyzed using a 2100 Bioanalyzer (Agilent) to assess DNA size distribution and size range. Fragmented DNA was ligated to Ion-compatible adaptors and library barcodes, followed by nick-repair to complete the linkage between adaptors and DNA inserts using the Ion Plus Fragment Library kit (Life Technologies). The adapter-ligated library

was size-selected for optimum length on 2% Agarose Gel Cassettes (Sage Science, Beverly, Mass., USA) using the Pippin Prep™ instrument (Sage Science). Library concentration was normalized using the Ion Library Equalizer™ Kit (Life Technologies). Next, the DNA library was clonally amplified onto Ion Sphere™ Particles generating template-positive ISPs using the Ion PGM™ Template OneTouch™ 2 200 Kit (Life Technologies) with the Ion OneTouch™ 2 Instrument (Life Technologies). Before proceeding to enrichment, quality assessment of non-enriched template-positive ISPs was performed using the Ion Sphere™ Quality Control assay kit (Life Technologies) and a Qubit® 2 Fluorometer instrument. The template-positive ISPs were then enriched using the Ion PGM™ Template OneTouch™ 2 200 Kit (Life Technologies) and Ion OneTouch™ ES instrument (Life Technologies) to eliminate template-negative ISPs and to denature DNA on template-positive ISPs. Using the Ion PGM™ 200 Sequencing v2 Kit (Life Technologies), enriched template ISPs were prepared for sequencing and loaded onto either Ion 314™ or Ion 316™ Chip v2 (Life Technologies) and run on the Ion PGM™ Sequencer (Life Technologies). Obtained sequences were then trimmed using Galaxy (<https://usegalaxy.org/>) NGS QC and Manipulation tools. Sequences were aligned and analyzed using Sequencher 5.2.2 (Genecodes) and CLC Genomics Workbench (CLCBio) software.

The following differences were observed between these two viruses (nucleotide positions are provided based on ASFV Georgia 2007/1, GenBank accession FR682468): (i) two nucleotide insertions, T at position 433 and A at position 441 in a non-coding segment of the genome; (ii) two nucleotide deletions, T at position 1602 and T at position 1603 in the MGF 360-1 L gene ORF resulting in a frameshift; (iii) a nucleotide deletion, T at position 1620 in the MGF 360-1 L gene ORF resulting in a frameshift; (iv) a nucleotide mutation, A to G at position 97391 resulting in a silent mutation in ORF B438L; (v) a nucleotide mutation, C to G at position 166192 resulting in a residue substitution (Ala to Pro) at residue position 85 in ORF E199L; and (vi) a nucleotide insertion, T at position 183303, a non-coding segment of the genome (Table 1). Second, a full-length genome comparison between ASFV Δ9GL and parental ASFV-G was performed. The DNA sequence assemblies of ASFV Δ9GL and ASFV-G revealed a deletion of 173 nucleotides in ORF B119L (9GL) corresponding with the introduced modification. The consensus sequence of the ASFV-G Δ9GL genome showed an insertion of 2324 nucleotides in ORF B119L corresponding to the p72-βGUS cassette sequence introduced to generate a 173 nucleotide deletion in the targeted gene. Besides the insertion of the cassette, only one additional difference was observed between ASFV-G Δ9GL and ASFV-G genomes, a G to C nucleotide mutation at position 36465 resulting in a residue substitution (Glu to Gln) at residue position 224 in ORF MGF 505-4R. In summary, ASFV-G Δ9GL virus did not accumulate any significant mutations during the process of homologous recombination and plaque purification.

TABLE 1

| Summary of differences between the full-length genome sequence of ASFV-G Δ9GL and the parental ASFV-G compared with ASFV Georgia07/1* | | | | |
|---|----------------------------|--------|-------------|--|
| Virus | | | | |
| NPN* | Type of Modification | ASFV-G | ASFV-G Δ9GL | |
| 433 | T insertion | + | + | |
| 411 | A insertion | + | + | |
| 1602 | MGF 360-1L TT deletion FS@ | + | + | |

TABLE 1-continued

| Summary of differences between the full-length genome sequence of ASFV-G Δ9GL and the parental ASFV-G compared with ASFV Georgia07/1* | | | | |
|---|-----------------------------|--------|-------------|--|
| Virus | | | | |
| NPN* | Type of Modification | ASFV-G | ASFV-G Δ9GL | |
| 1620 | MGF 360-1L T insertion FS | + | + | |
| 36465 | MGF 505-4R G to C Glu224Gln | - | + | |
| 97391 | B438L A to G SM# | + | + | |
| 166192 | E199L C to G Ala85Pro | + | + | |
| 183303 | T insertion in a NCR* | + | + | |

*Nucleotide Position Number (based on the sequence of ASFV Georgia 2007/1 isolate published by Chapman et al. 2011)

@Nucleotide modification causes frameshift in the corresponding ORF

#Nucleotide modification causes silent mutation

*Non-Coding Region

Example 4

Replication of ASFV-G Δ9GL in Primary Swine Macrophages

In vitro growth characteristics of ASFV-G Δ9GL were evaluated in primary swine macrophage cell cultures, the primary cell targeted by ASFV during infection in swine, and compared relative to parental ASFV-G in a multistep growth curve (FIG. 3). Cell cultures were infected at a MOI of either 0.1 or 0.01 and samples were collected at 2, 24, 48, 72 and 96 hours post-infection (hpi). ASFV-G Δ9GL virus displayed a growth kinetic significantly slower than parental ASFV-G virus (FIG. 3). Depending on the time point and MOI utilized to infect macrophages, the recombinant virus exhibited titers 10- to 10,000-fold lower relative to the parental virus. Therefore, and as observed with ASFV Malawi Lil-20/1 Δ9GL virus, deletion of the 9GL (B119L) gene significantly affects the ability of the virus to replicate in vitro in primary swine macrophage cell cultures.

Example 5

Assessment of ASFV-G Δ9GL Virulence in Swine

Animal experiments were performed under biosafety level 3 conditions in the animal facilities at PIADC following a protocol approved by the Institutional Animal Care and Use Committee.

ASFV-G Δ9GL was assessed for its virulence phenotype relative to the virulent parental ASFV-G virus using 80-90 pound commercial breed swine. Six pigs were inoculated intramuscularly (IM) either with 10^2 or 10^4 HAD₅₀ of either ASFV-G Δ9GL or ASFV-G virus. Clinical signs (anorexia, depression, fever, purple skin discoloration, staggering gait, diarrhea and cough) and changes in body temperature were recorded daily throughout the experiment. In protection experiments animals were IM inoculated with 10^3 HAD₅₀ and 28 days later IM challenged with 10^3 HAD₅₀ of parental virulent ASFV Georgia 2007 strain. Presence of clinical signs associated with the disease was performed as described earlier.

Deletion of the 9GL (B119L) gene from the genomes of ASFV isolates Malawi Lil-20/1 and Pretoriuskop/96/4 has been shown to drastically reduce virulence in swine (Lewis et al., supra; Neilan et al. 2004, supra). In those reports, IM inoculation of either recombinant deletion mutant at doses as high as 10^4 (Lewis et al., supra; Neilan et al. 2004, supra) or even 10^6 TCID₅₀ (Lewis et al., supra) only induced a

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transient rise in body temperature. Furthermore, animals inoculated with either Malawi Lil-20/1 or Pretoriuskop/96/4 Δ9GL viruses remained clinically normal after challenge with the corresponding virulent parental viruses.

Here, 80-90 pounds pigs inoculated via IM with 10^4 HAD₅₀ of ASFV-G exhibited increased body temperature ($>104^\circ$ F.) by 3 to 4 days post-infection. Pigs presented clinical signs associated with the disease including anorexia, depression, purple skin discoloration, staggering gait and diarrhea (Table 2). Signs of the disease aggravated progressively over time and animals either died or were euthanized in extremis by days 7 or 8 post-infection. Interestingly, animals inoculated via IM with 10^4 HAD₅₀ of ASFV-G Δ9GL developed clinical disease practically indistinguishable from that observed in animals inoculated via IM with 10^4 HAD₅₀ of parental ASFV-G being the only difference a slight delay in the appearance of fever. Animals inoculated IM with 10^2 HAD₅₀ of ASFV-G develop a clinical disease comparable in severity to that observed in animals infected with 10^4 HAD₅₀ with the exception that clinical signs appeared delayed in two days as well as the time of death was. Pigs inoculated via IM with 10^2 HAD₅₀ of parental ASFV-G developed a slightly delayed clinical disease relative to pigs inoculated with 10^4 HAD₅₀ of the same virus. Pigs presented a short period of fever starting by day 7 post-infection, with animals dying or euthanized in extremis around 8 to 9 days post-infection. Severity of the clinical signs observed in these animal were similar to those inoculated with the higher (10^4 HAD₅₀) dose. Conversely, pigs inoculated via IM with 10^2 HAD₅₀ of mutant ASFV-G Δ9GL did not present any signs of the clinical disease during the entire observation period (21 days). Therefore, the degree of virulence of ASFV-G Δ9GL virus when experimentally IM-inoculated into swine appears to depend on the amount of infectious virus used in the experimental inoculation.

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showed in FIG. 2A), viruses isolated from blood were tested by PCR using primers that target p72 (B646L), 9GL (B119L) and β-Gus genes. All four ASFV-G Δ9GL viruses isolated from blood of inoculated animals tested negative for parental ASFV-G. The 9GL (B119L) gene was not detected in these viruses, whereas amplification of p72 (B646L) and β-Gus genes was observed in all instances (FIG. 2B). Furthermore, PCR amplification followed by sequencing was conducted on blood-isolated viruses to assess the integrity of the p72-βGus cassette inserted in ASFV-G Δ9GL by homologous recombination. Obtained sequences revealed that the p72-βGus and both flanking regions were not modified in these viruses (data not shown). Since these data indicated the absence of contamination of the inoculum with parental ASFV-G, it was concluded that ASFV-G Δ9GL virus inoculated at high doses (10^4 HAD₅₀) is able to induce a clinical disease basically indistinguishable from that induced by the parental virus.

As previous studies with ASF Malawi Lil-20/1 and Pretoriuskop/96/4 and E70 had shown, a deletion within the 9GL (B119L) gene had resulted in attenuated ASF viruses (Δ9GL ASF Malawi Lil-20/1 and Δ9GL ASF Pretoriuskop/96/4 and E70) which did not cause disease at high doses (10^4 HAD₅₀) and were effective at 10^4 HAD₅₀, 10^3 HAD₅₀, and 10^2 HAD₅₀ for protection from pathogenic parental Malawi Lil-20/1 and Pretoriuskop/96/4 and E70 ASFV. However, the modification of the 9GL (B119L) gene of the highly virulent ASFV Georgia 2007 isolate, of Δ9GL ASFV-G, of the present invention, differs from the deletion of that obtained in the prior studies, i.e., a larger number of amino acids have been deleted in the recombinant ASFV-G and the deletion in of Δ9GL ASFV-G covers an area of the targeted 9GL (B119L) region that is different from the modified of Δ9GLASF Malawi Lil-20/1 and of Δ9GLASF Pretoriuskop/96/4 and E70 as shown in Table 3. Further, unlike Δ9GL

TABLE 2

| Virus | No. of Survivors/ Total | Mean Time to death (Days ± SD) | Fever | | |
|--------------------------|-------------------------|--------------------------------|----------------------------------|----------------------------------|--------------------------------|
| | | | No. of Days to onset (Days ± SD) | Duration No. of Days (Days ± SD) | Maximum Daily Temp (F. ° ± SD) |
| ASFV-G | 0/5 | 9.4 (1.22) | 7 (0.0) | 1.2 (0.82) | 106.7 (0.58) |
| 10^2 HAD ₅₀ | | | | | |
| ASFV-G Δ9GL | 10/10 | — | — | — | 103.0 (0.17) |
| 10^2 HAD ₅₀ | | | | | |
| ASFV-G | 0/10 | 7.25 (0.7) | 3.5 (0.76) | 3.75 (0.71) | 107 (0.47) |
| 10^4 HAD ₅₀ | | | | | |
| ASFV-G Δ9GL | 0/5 | 8.25 (1.6) | 5.25 (1.91) | 3.25 (0.46) | 106.5 (0.46) |
| 10^4 HAD ₅₀ | | | | | |

Viremia in experimentally inoculated animals was quantified at day 7 post-infection. Pigs inoculated with either 10^2 or 10^4 HAD₅₀ of virulent parental ASFV-G had virus titers in blood averaging 7.92 (SD=0.17) and 8.28 (SD=0.09) HAD₅₀/ml, respectively. Pigs inoculated with 10^4 HAD₅₀ of mutant ASFV-G Δ9GL had virus titers in blood averaging 2.44 (SD=0.2) HAD₅₀/ml, whereas pigs inoculated with 10^2 HAD₅₀ of mutant ASFV-G Δ9GL presented barely detectable virus titers in blood, averaging 1.97 (SD=0.1) HAD₅₀/ml. Therefore, despite a low titer in blood that might indicate limited replication in vivo, ASFV-G Δ9GL induces disease in pigs without reaching the viremia levels observed in animals inoculated with parental ASFV-G.

To rule out that the disease observed in the animals inoculated via IM with 10^4 HAD₅₀ of ASFV-G Δ9GL was caused due to contamination of the inoculum with remaining parental ASFV-G (undetectable by the PCR methodology

ASF Malawi Lil-20/1 and Δ9GL ASF Pretoriuskop/96/4 and E70, inoculation of Δ9GL ASFV-G at 10^4 HAD₅₀ causes disease and death; whereas, inoculation of Δ9GL ASFV-G at 10^3 HAD₅₀ and 10^2 HAD₅₀ does not.

TABLE 3

| Comparison of 9GL deletions in ASFV-G and Malawi Lil 20/1. | | |
|--|------------|-----------------|
| | ASFV-G | Malawi Lil 20/1 |
| Length of genome | 189,284 bp | 187,612 bp |

TABLE 3-continued

| Comparison of 9GL deletions in ASFV-G and Malawi Lil 20/1. | | | | |
|--|----------------|------------------------|---------------------|-------------------------|
| | Protein Length | | Deleted AA* | |
| | 9GL ASFV-G | 9GL Malawi Lil 20/1 | Δ9GL ASFV-G | Δ9GL Malawi Lil 20/1 |
| # of AA | 119 | 119 | 58 | 29 |
| Position: | — | — | 11-68 | 51-79 |
| Deleted AA | | | | |
| | Gene Length | | Deleted Nucleotides | |
| | 9GL ASFV-G | 9GL Malawi Lil 20/1 | Δ9GL ASFV-G | Δ9GL Malawi Lil 20/1 |
| # Nucleo-tides | 357 | 357 | 172 | 82 |
| Position: | — | — | 32 to 204 | 154 to 236 |
| Deleted Nucleo-tides | | | | |

*Amino Acids

Example 6

Protective Efficacy of ASFV-G Δ9GL Against Challenge with Parental ASFV-G

Since pigs inoculated via IM with 10^2 HAD₅₀ of ASFV-G Δ9GL survived the infection without signs of the disease, a group of animals (n=5) were infected with a slightly higher dose (10^3 HAD₅₀) of ASFV-G Δ9GL and challenged via IM with 10^3 HAD₅₀ of parental ASFV-G at day 28 post-inoculation (homologous challenge). Five naïve animals that were challenged using same route and dose served as non-inoculated/challenged control group. The five ASFV-G Δ9GL inoculated/challenged remained completely asymptomatic during all the observational period (21 days) with the exception of one animal showing a slight and transient rise in body temperature by day 8 pi. (Table 4). All the animals in the non-inoculated/challenged control group developed disease with a clinical course similar to that observed in animals inoculated with 10^2 or 10^4 HAD₅₀ of ASFV-G (see above). Therefore, sub-lethal doses of ASFV-G Δ9GL are able to induce protection against the presentation of clinical disease when challenged with the highly virulent parental virus.

TABLE 4

Swine survival and fever response in ASFV-G Δ9GL- infected animals challenged with parental ASFV-G viruses.*

| Virus | No. of Survivors/ Total | Fever | | | | |
|------------------------------------|----------------------------|--------------------------|--|--|--------------------------------------|--|
| | | Mean Time (Days ± SD) | No. of Days to onset (Days ± SD) | Duration No. of Days (Days ± SD) | Maximum Daily Temp (F. ° ± SD) | |
| ASFV-G 10^3 HAD ₅₀ | 5/5 | — | — | — | 106.2 (0.87) [#] | |
| Mock infected | 0/5 | 8.25 (0.6) | 3.5 (0.8) | 4.25 (0.9) | 106.1 (0.14) | |

*The animals IM infected with 10^3 HAD₅₀ of ASFV-G Δ9GL were IM challenged 21 days later with 10^3 HAD₅₀ of ASFV-G virus.[#]This temperature corresponds to the only animal to show a transient rise in temperature.

Example 7

Genetic DIVA Strategy—Real-Time RT-PCR

- 5 Total DNA was extracted from blood, serum and organ samples using the DNeasy mini kit (Qiagen), following the manufacturer's recommendations. Extracted DNA was subject to real time-PCR assay for detection and differentiation of ASFV-G and ASFV-GΔ9GL. The highly conserved full-length 9GL gene is present in the genome of wild-type ASFV-G and is detected in organs, blood and serum of infected pigs whereas the gene is not detected in pigs vaccinated with the vaccine comprising ASFV-GΔ9GL where a portion of the 9GL has been deleted.
- 10 15 ASFV-G DNA is detected by Sybr Green real time PCR using primer set: 9GL-For-191 5'GTAAGATAC-GAAAAGCGTG3' (SEQ ID NO:11) and 9GL-Rev-297 5'GACGCTCCTAGCTGGAA3' (SEQ ID NO:12); ASFV-GΔ9GL is not detected. Modifications in ASFV-GΔ9GL
- 20 25 DNA is detected by Sybr Green real time PCR using primer set: 9GL-For-127 5'GTTGTTATGGAACGCGAAG3' (SEQ ID NO:13) and GUS-Rev-366 5'GGGTTTCTACAG-GACGTAACA3' (SEQ ID NO:14) or primer set: GUS-TT-For 5'CTGTTGAATTACGTAAAGCATG3' (SEQ ID NO:15) and 9GL-Rev-351 5'CATTG GGGAC-CTAAATACTG3' (SEQ ID NO:16); but wild type ASFV-G DNA is not detected by these primer sets. Assays were run in parallel.

30 Samples from vaccinated animals were analyzed for differentiation and confirmation using the set of primers by means of Sybr Green real time-PCR. Samples (n=5) from vaccinated animals tested positive only to ASFV-GΔ9GL and negative for wild type ASFV-G. After challenge, samples (n=5) from vaccinated pigs tested positive to ASFV-G by real time-PCR. Non vaccinated control pigs (n=5) tested positive to ASFV-G by real time-PCR but tested negative for ASFV-GΔ9GL.

Example 8

ASFV 9GL (B119L) Gene is Highly Conserved

35 40 Sequence analysis of the 9GL (B119L) genes from several ASFV isolates obtained from various temporal and geographic origins, including those from both tick and pig sources, reveals a high degree of conservancy (FIG. 1). Isolates compared include those from ticks: Malawi Lil-20/1 (1983), Crocodile/96/1 (1996), Crocodile/96/3 (1996), Pretoriuskop/96/5 (1996), Pretoriuskop/96/4 (1996), Fairfield/96/1, and Wildebeeslaagte/96/1; domestic pig isolates:

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Georgia 2007/1 (2007), Killean 3, European-70 (1970), European-75 (1975), Kimakia (1964), Victoria Falls, La Granja (1963), Lisbon60 (1960), Spencer (1951), Tengani (1962), Zaire (1967) and Haiti 811 (1980); warthog isolate Uganda (1961); and bush pig isolate Lee (1955). Among these isolates amino acid identity for 9GL (B110L) ranges between 93 to 100%.

As a summary, results presented here demonstrated that genetic changes introduced in the 9GL gene of ASFV-G results in the ASFV-G Δ9GL mutated virus which in a vaccine induces a solid protection against the challenge in animals vaccinated 21 days earlier.

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All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

The foregoing description and certain representative embodiments and details of the invention have been presented for purposes of illustration and description of the invention. It is not intended to be exhaustive or to limit the invention to the precise forms disclosed. It will be apparent to practitioners skilled in this art that modifications and variations may be made therein without departing from the scope of the invention.

SEQUENCE LISTING

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<210> SEQ ID NO 1

<211> LENGTH: 189346

<212> TYPE: DNA

<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 1

| | | | | | | |
|-------------|--------------|-------------|-------------|-------------|------------|------|
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| tatggccgga | ccaatccatg | gctgcactta | aaaatatcaa | aaaagttaa | gttttgggcc | 120 |
| ggcggtaaaa | tttaaacctt | ttctggttga | tcttagcca | tgtatagctg | cgtatgttgg | 180 |
| tgccttatct | acatgctatt | ggcatctctg | atattcgac | taaagtgtca | tgttacaacc | 240 |
| gtcttatgcg | tgatTTTAT | ccaccttatt | ggccgaaagg | ccgccttgta | tttcctgtta | 300 |
| ggtggttgg | ccgtattcta | ctgggtggaa | gcagctatca | ataaaattta | atggctctca | 360 |
| cttaagatcc | ttgctgttaag | cgggcgttta | catactttga | tcaagaaaaa | aattatTTT | 420 |
| ggaccccccc | ccatgtttt | tacaaaatc | atataataaa | gtggcgacaa | tcaacatatt | 480 |
| aatcaaccac | agcattttat | gatgtgttaa | tcaacatata | ccatattaat | caaccacagc | 540 |
| atTTTatgat | gcgtcaatca | acatattatt | acggagacg | tcaatcaata | taatattgag | 600 |
| aacagcgact | tgataccgtg | tatgggttg | gcccggcat | gttggTTGta | acagcatttt | 660 |
| tcatcatcg | aagcttacaa | aagatATGta | taagatAGca | tattaatgtt | attaacagta | 720 |
| atataataaa | ggcgtagcta | tagatCTCA | cTTGGTAgA | ccaataatcc | atggTTGCGC | 780 |
| ttaaaaatac | caaaaaaaaaac | attaagTTTT | ggagggttaa | attggTTTT | caccattgg | 840 |
| aaagattatt | attctaaatg | tttACCCat | agatgtgaaa | caatgattct | tcatatatta | 900 |
| acatTTTTT | tgacttatac | tttCTTCat | ctagtaaggc | gttaattttt | tccggatctg | 960 |
| tcgtttttat | tgataaaaaga | gaagagtctg | gactgttaatt | ttaaataat | aagatattta | 1020 |
| ttaatATCCA | attattcgtt | tggctcgcta | tttCCatGCT | ctttcgaaa | gcatcagctc | 1080 |
| ctaaatctat | acaaaggaaat | aagttaacctt | cacaaaattt | cattaccgag | gtaatcattg | 1140 |
| cccgattaat | gtcagcccccc | aacataaaaac | aataatata | agttgtataa | ttacaatcat | 1200 |
| acatacaggc | caactgcac | atttcataa | tgtctatatt | tgtcttctct | ttgttataaa | 1260 |
| tttcatgaag | gtcaaagacg | ttgttataag | caacccaca | tattaaccgc | caatcttaa | 1320 |
| aatgactata | tcgttgataa | aaatatttgg | tggcttcagt | aagcttataat | agtatcgcca | 1380 |
| tactatacca | ataccttagtt | agcatttcgt | tgaatgaaat | attatccaaat | gtaaagttaa | 1440 |
| ttgataatgt | atctagttaa | ccaaaaatTC | ttaatttcag | ttgagcatta | tttaggaaaa | 1500 |
| ggggattatac | agataataat | tcatggcata | gaataatatt | actgctagtt | ttaacatact | 1560 |
| gtacattata | aaatatttct | aaaattttat | tttcaactaa | agcttccctc | gcacctaact | 1620 |

-continued

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| | | 20 | | | | | 25 | | | | 30 | | | | |

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|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
| Tyr | Gly | Lys | Thr | Asp | Phe | Ile | Gly | Ala | Trp | Ser | Ser | Leu | | | |
| | 50 | | | | 55 | | | | | 60 | | | | | |

<210> SEQ ID NO 5

<211> LENGTH: 20

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 5

tagagatgac caggctccaa

20

<210> SEQ ID NO 6

<211> LENGTH: 23

<212> TYPE: DNA

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 6

gttgcattgg ggacctaaat act 23

<210> SEQ ID NO 7
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 7

gacggcctgt gggcatt 17

<210> SEQ ID NO 8
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 8

gcgatggatt ccggcat 17

<210> SEQ ID NO 9
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 9

gtcttattgc taacgatggg aag 23

<210> SEQ ID NO 10
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 10

ccaaaggtaa gcttgttcc caa 23

<210> SEQ ID NO 11
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 11

gtaagatacg aaaaggcgta 20

<210> SEQ ID NO 12
<211> LENGTH: 17
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 12

gacgctccta gctggaa 17

-continued

<210> SEQ ID NO 13
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 13

gttgttatgg aacgcgaag

19

<210> SEQ ID NO 14
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 14

gggtttctac aggacgtaac a

21

<210> SEQ ID NO 15
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 15

ctgttgaatt acgttaagca tg

22

<210> SEQ ID NO 16
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Chemically Synthesized

<400> SEQUENCE: 16

cattggggac ctaaatactg

20

<210> SEQ ID NO 17
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 17

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Leu | His | Trp | Gly | Pro | Lys | Phe | Trp | Arg | Thr | Leu | His | Leu | Tyr | Ala |
| 1 | | | 5 | | | 10 | | 15 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Phe | Phe | Ser | Asp | Thr | Pro | Gly | Trp | Lys | Glu | Lys | Tyr | Glu | Ala | Ile |
| 20 | | | | | | 25 | | 30 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Trp | Ile | Leu | Asn | Phe | Ile | Glu | Ser | Leu | Pro | Cys | Thr | Met | Cys | Arg |
| 35 | | | | | | 40 | | 45 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | His | Ala | Phe | Ser | Tyr | Leu | Thr | Lys | Asn | Pro | Leu | Thr | Leu | Asn | Asn |
| 50 | | | | | | 55 | | 60 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Glu | Asp | Phe | Gln | Tyr | Trp | Thr | Phe | Ala | Phe | His | Asn | Asn | Val | Asn |
| 65 | | | | | | 70 | | 75 | | | 80 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lys | Arg | Leu | Asn | Lys | Lys | Ile | Ile | Ser | Trp | Ser | Glu | Tyr | Lys | Asn | Ile |
| 85 | | | | | | 90 | | 95 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Glu | Gln | Ser | Ile | Leu | Asn | Thr | Ile | Glu | Tyr | Gly | Lys | Thr | Asp | Phe |
| 100 | | | | | | 105 | | 110 | | | | | | | |

Ile Gly Ala Trp Ser Ser Leu

-continued

115

<210> SEQ ID NO 18
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 18

```

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ala Leu His Leu Tyr Ala
1           5          10          15

Ile Phe Phe Ser Asp Ala Pro Gly Trp Lys Glu Lys Tyr Glu Ala Ile
20          25          30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Arg
35          40          45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50          55          60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65          70          75          80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85          90          95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100         105         110

Ile Gly Ala Trp Ser Ser Leu
115

```

<210> SEQ ID NO 19
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 19

```

Met Leu His Trp Gly Pro Lys Phe Trp Arg Ala Leu His Leu Tyr Ala
1           5          10          15

Ile Phe Phe Ser Asp Ala Pro Asn Trp Lys Glu Lys Tyr Glu Ala Ile
20          25          30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Met Cys Arg
35          40          45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
50          55          60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
65          70          75          80

Lys Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
85          90          95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
100         105         110

Ile Gly Ala Trp Ser Ser Leu
115

```

<210> SEQ ID NO 20
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 20

```

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ala Leu His Leu Tyr Ala
1           5          10          15

Ile Phe Phe Ser Asp Thr Pro Gly Trp Lys Glu Lys Tyr Glu Ala Ile
20          25          30

```

-continued

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
 35 40 45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
 50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
 65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
 85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
 100 105 110

Ile Gly Ala Trp Ser Ser Leu
 115

<210> SEQ ID NO 21
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 21

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ala Leu His Leu Tyr Ala
 1 5 10 15

Ile Phe Phe Ser Asp Ala Pro Gly Trp Lys Glu Lys Tyr Glu Ala Ile
 20 25 30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
 35 40 45

His His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
 50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
 65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
 85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
 100 105 110

Ile Gly Ala Trp Ser Ser Leu
 115

<210> SEQ ID NO 22
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 22

Met Leu His Trp Gly Pro Lys Tyr Trp Arg Ser Leu His Leu Tyr Ala
 1 5 10 15

Ile Phe Phe Ser Asp Ala Pro Ser Trp Lys Glu Lys Tyr Glu Ala Ile
 20 25 30

Gln Trp Ile Leu Asn Phe Ile Glu Ser Leu Pro Cys Thr Arg Cys Gln
 35 40 45

Arg His Ala Phe Ser Tyr Leu Thr Lys Asn Pro Leu Thr Leu Asn Asn
 50 55 60

Ser Glu Asp Phe Gln Tyr Trp Thr Phe Ala Phe His Asn Asn Val Asn
 65 70 75 80

Asn Arg Leu Asn Lys Lys Ile Ile Ser Trp Ser Glu Tyr Lys Asn Ile
 85 90 95

Tyr Glu Gln Ser Ile Leu Lys Thr Ile Glu Tyr Gly Lys Thr Asp Phe
 100 105 110

-continued

Ile Gly Ala Trp Ser Ser Leu
115

<210> SEQ ID NO 23
<211> LENGTH: 119
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 23

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Leu | His | Trp | Gly | Pro | Lys | Tyr | Trp | Arg | Ser | Leu | His | Leu | Tyr | Ala |
| 1 | | | | 5 | | | | 10 | | | | 15 | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ile | Phe | Phe | Ser | Asp | Ala | Pro | Ser | Trp | Lys | Glu | Lys | Tyr | Glu | Ala | Ile |
| 20 | | | | | 25 | | | | 30 | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Trp | Ile | Leu | Ser | Phe | Ile | Glu | Ser | Leu | Pro | Cys | Thr | Arg | Cys | Gln |
| 35 | | | | | 40 | | | | 45 | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| His | His | Ala | Phe | Ser | Tyr | Leu | Thr | Lys | Asn | Pro | Leu | Thr | Leu | Asn | Asn |
| 50 | | | | | 55 | | | 60 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Glu | Asp | Phe | Gln | Tyr | Trp | Thr | Phe | Ala | Phe | His | Asn | Asn | Val | Asn |
| 65 | | | | | 70 | | | 75 | | | 80 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Arg | Leu | Asn | Lys | Lys | Ile | Ile | Ser | Trp | Ser | Glu | Tyr | Lys | Asn | Ile |
| 85 | | | | | 90 | | | 95 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Tyr | Glu | Gln | Ser | Ile | Leu | Lys | Thr | Ile | Glu | Tyr | Gly | Lys | Thr | Asp | Phe |
| 100 | | | | | 105 | | | 110 | | | | | | | |

Ile Gly Ala Trp Ser Ser Leu
115

<210> SEQ ID NO 24
<211> LENGTH: 58
<212> TYPE: PRT
<213> ORGANISM: African swine fever virus

<400> SEQUENCE: 24

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Leu | His | Leu | Tyr | Ala | Ile | Phe | Phe | Ser | Asp | Ala | Pro | Ser | Trp | Lys |
| 1 | | | | | 5 | | | 10 | | | 15 | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Lys | Tyr | Glu | Ala | Ile | Gln | Trp | Ile | Leu | Asn | Phe | Ile | Glu | Ser | Leu |
| 20 | | | | | 25 | | | 30 | | | | | | | |

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Cys | Thr | Arg | Cys | Gln | His | His | Ala | Phe | Ser | Tyr | Leu | Thr | Lys | Asn |
| 35 | | | | | 40 | | | 45 | | | | | | | |

| | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Thr | Leu | Asn | Asn | Ser | Glu | Asp | Phe |
| 50 | | | | | 55 | | | | |

We claim:

1. A recombinant ASFV-G (African Swine Fever Virus-⁵⁰ Georgia 2007 isolate) mutant, the mutant ASFV-G Δ9GL virus, comprising cDNA (SEQ ID NO: 3) encoding a mutant ASFV-G Δ9GL protein wherein the mutant cDNA comprises a deletion of 172 nucleotides resulting in a mutant 9GL protein comprising 58 fewer amino acids than the non-mutated, wild-type 9GL protein of ASFV-G, amino acids #11 to #68 being deleted.

2. A vaccine composition against ASFV-G wherein said vaccine composition comprises the recombinant mutant ASFV-G Δ9GL virus according to claim 1.

3. A method for the protection of swine against African Swine Fever Virus-Georgia 2007 isolate (ASFV-G), comprising administering to swine a live attenuated ASFV-G Δ9GL vaccine comprising a recombinant mutant ASFV-G Δ9GL virus according to claim 1 in an amount effective to protect said swine from clinical ASF-G.⁵⁵

4. The method of claim 3 wherein the amount effective to protect said swine from clinical ASF-G is a vaccine comprising 10³ HAD₅₀ of ASFV-G Δ9GL virus.

* * * * *